

AD _____

Award Number: DAMD17-00-1-0480

TITLE: Development of Lauimalide-Based Microtubule-Stabilizing
Agents: New Chemistry for the Treatment of Breast Cancer

PRINCIPAL INVESTIGATOR: Bradley S. Davidson, Ph.D.

CONTRACTING ORGANIZATION: Utah State University
Logan, Utah 84322-1415

REPORT DATE: July 2003

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

20040513 039

REPORT DOCUMENTATION PAGEForm Approved
OMB No. 074-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE July 2003	3. REPORT TYPE AND DATES COVERED Annual (1 Jul 02-30 Jun 03)	
4. TITLE AND SUBTITLE Development of Laulimalide-Based Microtubule-Stabilizing Agents: New Chemistry for the Treatment of Breast Cancer			5. FUNDING NUMBERS DAMD17-00-1-0480	
6. AUTHOR(S) Bradley S. Davidson, Ph.D.				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Utah State University Logan, Utah 84322-1415 E-Mail: davidson@cc.usu.edu			8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012			10. SPONSORING / MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES				
12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited				12b. DISTRIBUTION CODE
13. ABSTRACT (Maximum 200 Words) <p>Through a collaborative research program involving Utah State University and the Cancer Research Center of Hawaii, we have discovered that the sponge-derived macrolides laulimalide and isolaulimalide are potent cytotoxins with paclitaxel-like antimicrotubule-stabilizing activity. Laulimalide is a potent inhibitor of cellular proliferation with an IC₅₀ in the low nanomolar range and it maintains activity against a drug resistant, P-glycoprotein over-expressing ovarian cancer cell line. Laulimalide represents a lead compound for new class of microtubule-stabilizing agents with activities that may prove therapeutically useful for the treatment of breast cancer. The aim of this project is to utilize a combinatorial solid-phase synthetic approach for the construction of a library of laulimalide analogs for structure activity relationship (SAR) studies.</p> <p>In an effort to discover a new chemotherapeutic agent for the treatment of breast cancer we propose to do the following: 1) transfer our current solution-phase synthetic approach to solid phase, 2) using a split and pool strategy, prepare 260 laulimalide analogs, 3) test laulimalide analogs for microtubule-stabilizing activity, cytotoxicity against both drug-sensitive and drug-resistant breast celllines, and 4) submit active analogs to the NCI for screening in the 60-cell line.</p> <p>Work to-date has been concentrated on the adaptation of our solution phase laulimalide synthetic methods for efficient conversion to the solid phase. Because of difficulties adding fragment C, a new retrosynthetic approach has been developed and is now being explored. It has the advantage that the coupling of fragment C to the resin-bound material will not involve the formation of a new asymmetric center, thus simplifying the reaction. Fragment B analogs have been prepared and coupled using a Julia-Kocienski type reaction to an appropriate aldehyde and the first two laulimalide analogs have been screened for biological activity..</p>				
14. SUBJECT TERMS No subject terms provided.				15. NUMBER OF PAGES 8
				16. PRICE CODE
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited	

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18
298-102

Table of Contents

Cover	1
SF 298	2
Table of Contents	3
Introduction	4
Body	4
Key Research Accomplishments	7
Reportable Outcomes	7
Conclusions	7
References	8
Appendices	NA

Introduction

In 1999 we reported that the marine macrolides laulimalide (**1**) is a new Taxol-like microtubule-stabilizing agents with activity against drug resistant cancer cells,¹ It therefore represents a new class of microtubule-stabilizing agent with activities that may prove therapeutically useful. The purpose of the proposed study is to produce an effective new agent for the treatment of breast cancer. With the goal of discovering a new chemotherapeutic agent for the treatment of breast cancer, we proposed to do the following: (a) transfer our current solution-phase synthetic technology to the solid phase; (b) using a split-and-pool strategy, prepare a library of up to 260 laulimalide analogs that are designed for 1) improved stability, 2) ease of synthesis, and 3) structural diversity; (c) screen laulimalide analogs for tubulin polymerization promoting activity and cytotoxicity toward drug-sensitive and drug-resistant breast cancer cell lines; and (d) submit active analogs to the NCI for screening in the 60-cell line assay. Our future goals include the selection of the most promising analogs for scale-up synthesis and *in vivo* testing.

Body

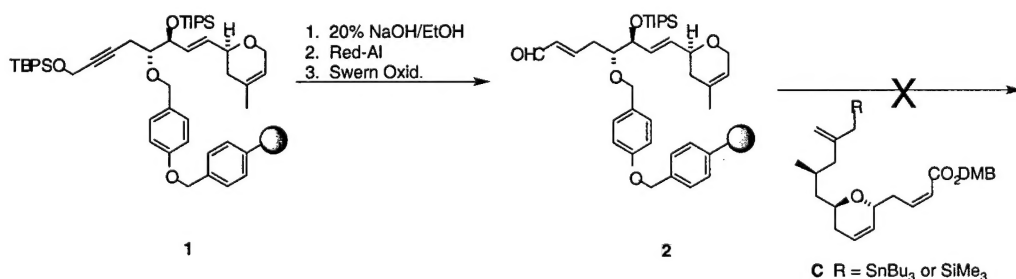
Research accomplishments for year 3 are described below for each of the Tasks in the approved Statement of Work.

Task 1. To adapt solution-phase synthetic technology to use on the solid phase (months 1-12)

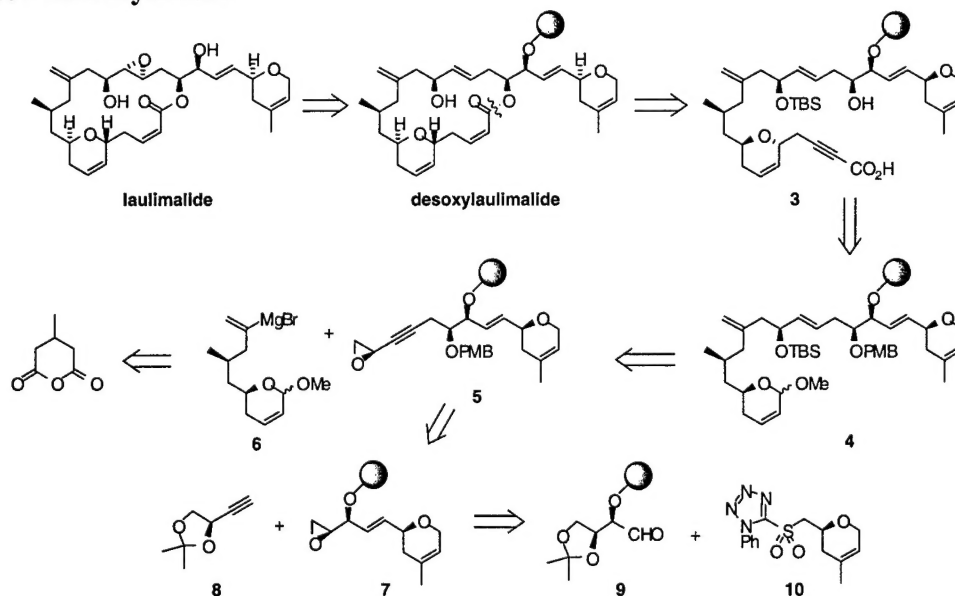
- Establish conditions for linking compounds to resin and for cleaving products from resin
- Optimize reaction times/conditions for solid phase, starting with conditions established for solution phase
- Synthesize laulimalide on solid phase

Task 1 is, unfortunately, still consuming much of our effort. Two crucial steps have presented difficulties: 1) the conversion of compound **1** to **2** (**10** to **11** in year 2 report), which been accomplished only in low yields and 2) the addition of major fragment **C** to **2** (represented in scheme 1 for the solid-phase) could not be accomplished in solution under conditions that would be sufficiently robust and generally applicable for the use on solid phase with a variety of analog fragments. The difficulty with the conversion from **1** to **2** results from insufficient stability of the linker to the conditions needed for removal of the *tert*-butyldiphenylsilyl (TBPS) protecting group. While this step works without difficulty in the solution phase, it has been unsatisfactory when applied to the solid phase synthesis. Because the TBPS group proved inert to the NaOH/MeOH, conditions that readily remove it in solution, we made two modifications. First we substituted the more reactive KOH for NaOH and we substituted EtOH for MeOH, which improved the swelling of the resin. Unfortunately, the reduction of the propargyl alcohol to the allylic alcohol with Red-Al was also problematic.

Scheme 1.



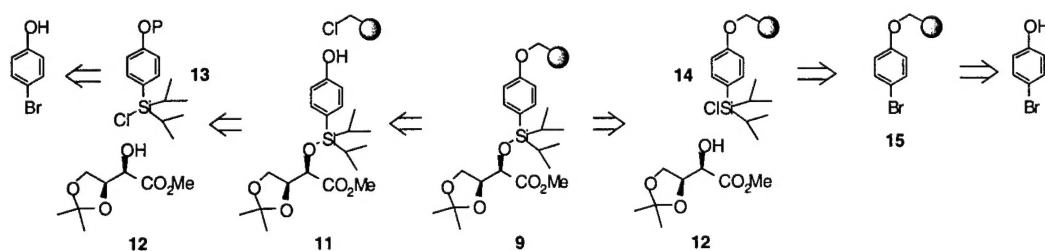
Scheme 2. New Retrosynthesis



5

to the silyl chloride was unsuccessful. We are not trying a *tert*-butyldimethylsilyl group, with the hope of selectively removing it in the presence of the silyl linker. Alternatively, The linker could be prepared on the resin from **14** and **15**.

Scheme 3. Linker Synthesis.

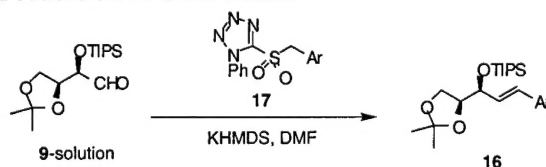


Task 2. Using a split-and-pool strategy, prepare a library of up to 260 laulimalide analogs that are designed for 1) improved stability, 2) ease of synthesis, and 3) structural diversity.

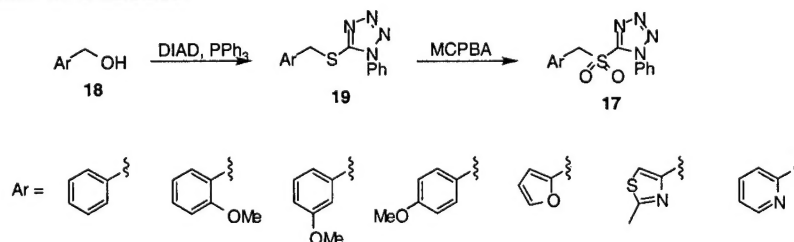
- Synthesize subunits **A2**, **B1-B10**, **C1-C15**. (months 1-12)
- Adapt solid phase reactions for use in micro-reactors (months 6-12)
- Phase I combinatorial synthesis of 60 laulimalide analogs (months 13-18)
- Phase II combinatorial synthesis of 200 laulimalide analogs (months 24-36)

Subunit synthesis has continued during year 3. While we had previously prepared **B** subunits as phosphonate esters with the goal of gaining the desired side chains with a *trans* geometry, attempts to carry out the Horner-Emmons type of reaction were unsuccessful under using a wide range of bases and solvents, often leading to decomposition of the aldehyde. Therefore, we renewed our attempts to prepare the sulfone reagents to be used in Julia-Kocienski reactions (see Scheme 4), analogous to our solution-phase work. To this effect, arylmethyl alcohols were coupled with 1-phenyl-5-mercaptopentazole under Mitsunobu conditions to give sulfides that need to be oxidized to the corresponding sulfones. Previously, attempts to carry out the oxidation using a variety of reagents, including ammonium molybdate- H_2O_2 , H_2O_2 -MeOH, oxone, and magnesium monoperoxyphthalate, gave only low yields using a variety of oxidizing agents. Fortunately, oxidation with *m*-chloroperoxybenzoic acid cleanly provides the desired sulfones, facilitating the preparation of the necessary reagents (Scheme 5).

Scheme 4. Propose Strategy for Addition of Side Chain.

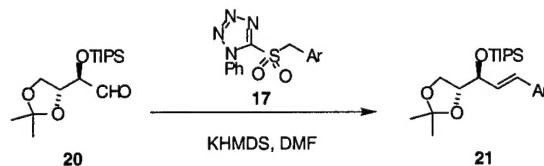


Scheme 5. Preparation of Sulfones.



Once prepared, coupling of the sulphone reagents **17** with aldehyde **20** yielded the corresponding products **21**. For Ar = Ph, a 74% yield was obtained, with a 5:1 ratio of *trans*:*cis*. The reactions were similarly successful with the methoxyphenyl analogs and with the furfuryl derivative, however, the thiazole and pyridine analogs have not yet been prepared.

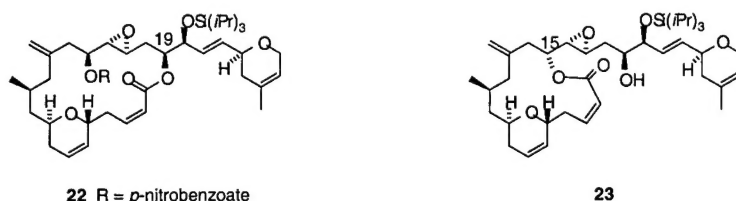
Scheme 6.



Task 3. Screen laulimalide analogs for tubulin polymerization promoting activity and cytotoxicity toward drug-sensitive and drug-resistant breast cancer cell lines.

- Establish benchmark activities for laulimalide in assays (months 13-18)
- Test Phase I combinatorial library (months 19-24)
- Test Phase II combinatorial library (months 25-36)

The first laulimalide analogs prepared as part of this study (**22** and **23**) were submitted to Dr. Mooberry for biological testing, but were shown to be inactive.



Task 4. Submit active analogs to the NCI for screening in the 60-cell line assay (months 19-36)

No progress yet.

Key Research Accomplishments

- Improved strategy for the preparation of laulimalide analogs.
- Preparation of subunits **B** and their successful coupling to an appropriate aldehyde (**20**).
- Testing of the first two laulimalide analogs.

Reportable Outcomes

Meeting Presentation

Bradley S. Davidson, "The Microtubule-Stabilizing Agent Laulimalide: Biological Activities And Synthetic Approaches, Thailand Research Fund-RGJ Meeting, Pattaya City, Thailand, April 2003.

Conclusions

During year 3, because of difficulties transferring the solution-phase synthetic route to the solid-phase, work has shifted to modifying the overall strategy and designing a new solid phase linker. Improved substructure **B** fragments were prepared and were demonstrated to couple with an appropriate aldehyde. The first laulimalide analogs prepared in this project were tested for microtubule-stabilizing activity, but were inactive.

References

1. Mooberry, S.L.; Tien, G.; Plubrukarn, A.; Davidson, B.S. Laulimalide and Isolaulimalide, New Paclitaxel-Like Microtubule-Stabilizing Agents. *Cancer Res.* **1999**, *59*, 653.
2. Mulzer, J.; Ohler, E. Microtubule-Stabilizing Marine Metabolite Laulimalide and Its Derivatives: Synthetic Approaches and Antitumor Activity. *Chem. Rev.* **2003**, *103*, 3753.
3. Ghosh, A.K.; Wang, Y.; Kim, J.T. Total Synthesis of Microtubule-Stabilizing Agent (—)-Laulimalide. *J. Org. Chem.* **2001**, *66*, 8973.